

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A multi-component detection system comprising:
  - i). a first agent comprising a first interacting group coupled directly or indirectly to a first tag, which first tag emits light of a first wavelength upon activation by a substrate or energy source, which produces a first activated tag;
  - ii). a second agent comprising a second interacting group coupled directly or indirectly to a second tag, which second tag can accept the energy from the first tag when the first and second interacting groups are associated and an appropriate substrate or energy source for the first tag is present thereby producing a second activated tag that emits light of a second wavelength;
  - iii). a third agent comprising a third interacting group coupled directly or indirectly to a third tag that can accept the energy from the first activated tag when the first and third interacting groups are associated and an appropriate substrate or energy source for the first tag is present to produce a third activated tag that emits light of a third wavelength;
  - iv). an appropriate substrate or energy source to activate the first tag, and
  - v). a means of detecting said emitted light.
2. A multi-component detection system comprising:
  - i). a first agent comprising a first interacting group coupled directly or indirectly to a first tag, which first tag emits light of a first wavelength upon activation by a substrate or energy source which produces a first activated tag;
  - ii). a second agent comprising a second interacting group coupled directly or indirectly to a second tag, which second tag can accept the energy from the first tag in i) when the first and second interacting

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groups are associated and an appropriate substrate or energy source for the first tag in i) is present thereby producing a second activated tag that emits light of a second wavelength;

5                   iii). a third agent comprising a third interacting group coupled directly or indirectly to a third tag that can accept the energy from the second activated tag in ii) when the first, second and third interacting groups are associated and an appropriate substrate or energy source for the first tag in i) is present to produce a third activated tag that emits light of a third wavelength, but said third tag is not substantially activated by the first activated tag in i) when only the first and third interacting groups are

10                  15 associated;

iv). an appropriate substrate or energy source to activate the tag in i); and

v). a means of detecting said emitted light.

20           3.           A multi-component detection system comprising:

i). a first agent comprising a first interacting group coupled directly or indirectly to a first tag, which first tag emits light of a first wavelength upon activation by a substrate or energy source

25                  which produces a first activated tag;

ii). a second agent comprising a second interacting group coupled directly or indirectly to a second tag, which second tag can accept the energy from the first tag in i) when the first and second interacting groups are associated and an appropriate substrate or energy source for the first tag in i) is present thereby producing a second activated tag that emits light of a second wavelength;

iii). a third agent comprising a third interacting group coupled directly or indirectly to a third tag that can accept the energy from the first activated tag in i) when the first and third interacting

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groups are associated and an appropriate substrate or energy source for the first tag in i) is present and that can accept the energy from the second activated tag in ii) when the second and third interacting groups are

5 associated and an appropriate substrate or energy source for the second tag in ii) is present to produce a third activated tag that emits light of a third wavelength;

iv). an appropriate substrate or energy source to activate the tags in i) and ii); and

10 v). a means of detecting said emitted light.

4. A multi-component detection system comprising:

i). a first agent comprising a first interacting group coupled directly or indirectly to a first tag, which first tag emits light of a first wavelength upon activation by a substrate or energy source which produces a first activated tag;

15 ii). a second agent comprising a second interacting group coupled directly or indirectly to a second tag, which second tag can accept the energy from the first tag in i) when the first and second interacting groups are associated and an appropriate substrate or energy source for the first tag in i) is present thereby producing a second activated tag that emits light of a second wavelength;

20 iii). a third agent comprising a third interacting group coupled directly or indirectly to a third tag consisting of a non-fluorescent quencher molecule that can accept the energy from:

25 a). the first activated tag when the first and third interacting groups are associated; and/or

30 b). the second activated tag when the second and third interacting groups are associated; and an appropriate substrate or energy source for the first and/or second tag is present, whereby the light emission from the first and/or second activated tag is decreased;

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iv). an appropriate substrate or energy source to activate the tags in i) and ii); and  
v). a means of detecting said emitted light.

5 5. A multi-component detection system comprising:

- i). a first agent comprising a first interacting group coupled directly or indirectly to a first tag, which first tag emits light of a first wavelength upon activation by a substrate or energy source 10 which produces a first activated tag;
- ii). a second agent comprising a second interacting group coupled directly or indirectly to a second tag, which second tag emits light of a second wavelength upon activation by a substrate or energy 15 source, which produces a second activated tag;
- iii). a third agent comprising a third interacting group coupled directly or indirectly to a third tag, which third tag can accept the energy from the first activated tag when the first and third interacting 20 groups are associated and an appropriate substrate or energy source for the first tag is present to produce a third activated tag that emits light of a third wavelength;
- iv). a fourth agent comprising a fourth interacting group coupled directly or indirectly to a fourth tag, which fourth tag can accept the energy from the second activated tag when the second and fourth interacting groups are associated and an appropriate substrate or energy source for the second tag is present 25 to produce a fourth activated tag that emits light of a fourth wavelength;
- v). an appropriate substrate or energy source to activate the first and second tags, and
- vi). a means of detecting said emitted light.

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6. A multi-component detection system comprising:

- i). a first agent comprising a first

interacting group coupled directly or indirectly to a first tag, which first tag emits light of a first wavelength upon activation by a substrate or energy source, which produces a first activated tag;

5           ii). a second agent comprising a second interacting group coupled directly or indirectly to a second tag, which second tag can accept the energy from the first tag when the first and second interacting groups are associated and an appropriate substrate or energy

10          source for the first tag is present thereby producing a second activated tag that emits light of a second wavelength;

15          iii). one or more further agents comprising one or more further interacting groups coupled directly or indirectly to one or more further tags that can accept the energy from the first activated tag when the first and one or more further interacting groups are associated and an appropriate substrate or energy source for the first tag is present to produce one or more further activated tags

20          25       that emit light of one or more further wavelengths, wherein said further wavelengths are different to the first or second wavelengths;

iv). an appropriate substrate or energy source to activate the first tag, and

v). a means of detecting said emitted light.

7.          A system according to any one of claims 1 to 6, wherein the interacting group is selected from the group consisting a compounds, proteins, protein domains, protein loops, protein termini, peptides, hormones, protein-lipid complexes, lipids, carbohydrates, carbohydrate-containing compounds, nucleic acids, oligonucleotides, pharmaceutical agents, pharmaceutical drug targets, antibodies, antigenic substances, viruses, bacteria, and cells.

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8.          A system according to any one of claims 1 to 6, wherein the interacting group is selected from the group

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consisting of carbohydrates, proteins, drugs, chromophores, antigens, chelating compounds, molecular recognition complexes and combinations thereof.

5 9. A system according to claim 7, wherein the nucleic acid molecule comprises DNA including single-stranded and double-stranded DNA, RNA including heterogeneous ribonucleic acid (hnRNA), transfer ribonucleic acid (tRNA), messenger ribonucleic acid (mRNA), or ribosomal ribonucleic acid (rRNA).

10. 10. A system according to any one of claims 1 to 9, wherein external stimuli are applied to directly or indirectly modulate the association of interacting groups.

15 11. A system according to claim 10, wherein the external stimuli are reagents comprising organic and inorganic molecules, proteins, nucleic acids, carbohydrates, lipids, ligands, drug compounds, agonists, 20 antagonists, inverse agonists or compounds.

12. A system according to claim 10, wherein the external stimuli are changes of conditions including temperature, ionic strength or pH.

25 13. A system according to any one of claims 1 to 12, wherein the detection tag is selected from the group consisting of a bioluminescent protein, a fluorescent protein, a fluorescent moiety or a non-fluorescent 30 quencher.

14. A system according to claim 13, wherein the bioluminescent protein is selected from the group consisting of luciferase, galactosidase, lactamase, 35 peroxidase or any protein capable of luminescence in the presence of a suitable substrate.

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15. A system according to claim 13, wherein the fluorescent protein selected from the group consisting of green fluorescent protein (GFP) or variants thereof, blue fluorescent variant of GFP (BFP), cyan fluorescent variant 5 of GFP (CFP), yellow fluorescent variant of GFP (YFP), enhanced GFP (EGFP), enhanced CFP (ECFP), enhanced YFP (EYFP), GFPs65T, Emerald, Topaz, GFPuv, destabilised EGFP (dEGFP), destabilised ECFP (dECFP), destabilised EYFP (dEYFP), HcRed, t-HcRed, DsRed, DsRed2, t-dimer2, t- 10 dimer2(12), mRFP1, pocilloporin, Renilla GFP, Monster GFP, paGFP, Kaede protein and kindling protein, Phycobiliproteins and Phycobiliprotein conjugates including B-Phycoerythrin, R-Phycoerythrin and Allophycocyanin or any other protein capable of 15 fluorescence.

16. A system according to claim 13, wherein the fluorescent moiety is selected from the group consisting of Alexa Fluor dyes and derivatives, Bodipy dyes and 20 derivatives, Cy dyes and derivatives, fluorescein and derivatives, dansyl, umbelliferone, fluorescent and luminescent microsheres, fluorescent nanocrystals, Marina Blue, Cascade Blue, Cascade Yellow, Pacific Blue, Oregon Green and derivatives, Tetramethylrhodamine and 25 derivatives, Rhodamine and derivatives, Texas Red and derivatives, rare earth element chelates or any combination or derivative thereof or any other molecule with fluorescent properties.

30 17. A system according to claim 13, wherein the non-fluorescent quencher is selected from the group consisting of dabcyl, non-fluorescent pocilloporins, QSY-7, QSY-9, QSY-21, QSY-35, BHQ-1, BHQ-2, BHQ-3 or any known non-fluorescent chromophore with the ability to absorb light 35 and to quench fluorescence and/or luminescence.

18. A system according to any one of claims 1 to 6

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wherein the activation energy is generated by a light source including lasers, Hg-lamps, Xe-lamps and halogen lamps or any light emitting device, and the generated light is restricted to a specific wavelength or wavelength range by appropriate means including laser lines, bandpass filters, shortpass filters, monochromators or any device to spectrally restrict the emitted light.

5 18. A system according to any one of claims 1 to 6, wherein the first activation energy is generated from a suitable enzyme substrate selected from the group consisting of coelenterazine and luciferin or a derivative derived therefrom.

10 15. A system according to claim 3, wherein sequentially the first detection tag is activated and the light emitted from the first, second and third detection tag is detected and then the second detection tag is activated and the light emitted from the second and third 20 detection tag is detected.

15 20. A system according to claim 4, wherein sequentially the first detection tag is activated and the light emitted from the first, second detection tag is detected and then the second detection tag is activated and the light emitted from the second detection tag is 25 detected.

20 21. A system according to claim 19 and 20, wherein the sequence of activation and detection is repeated over time to yield temporal information.

25 22. A system according to any one of claims 1 to 21, wherein the interacting group and tag are coded for in a fusion protein construct.

30 23. A recombinant DNA encoding a fusion protein

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construct according to claim 22.

24. A fusion gene that comprises a recombinant DNA according to claim 23.

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25. A DNA cassette comprising a promoter operably linked to one or more fusion protein genes according to any one of claims 22 to 24.

10 26. A vector comprising a fusion gene according to claim 25.

27. A host cell transiently or stably transformed or transfected by a vector according to claim 26.

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28. A host cell according to claim 27, wherein the cell is human, mammalian, insect, plant, bacterial, or yeast.

20 29. A vector according to claim 26, wherein the gene construct is under the control of a constitutive promoter.

30. A vector according to claim 26, wherein the gene construct is under the control of an inducible promoter.

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31. A vector according to claim 29, wherein the constitutive promoter is selected from the group consisting of CMV, SV40, RSV, EF-1 a, Tk, and AdML, when the cell to be transformed or transfected is mammalian.

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32. A vector according to claim 29, wherein the constitutive promoter is selected from the group consisting of T7, AraBAD, trc, pL, tac, and lac, when the cell to be transformed or transfected is a bacterial cell.

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33. A vector according to claim 29, wherein the constitutive promoter is selected from the group

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consisting of nmt1, gall, gal10, TEF1, AOX1, GAP, and ADH1, when the cell to be transformed or transfected is a yeast cell.

5 34. A virus comprising a fusion gene according to claim 25.

35. A host cell infected by a virus according to claim 34.

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36. Use of a multi-component detection system according to any one of claims 1 to 22, as an apoptotic sensor, wherein at least one of said agents is a caspase cleavage site.

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37. Use of a multi-component detection system according to any one of claims 1 to 22 to detect kinase activity, wherein at least one of said agents is a phosphorylation site.

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38. Use of a multi-component detection system according to any one of claims 1 to 22 to detect energy transfer in cell free systems.

25 39. Use of a multi-component detection system according to any one of claims 1 to 22 in a host cell by introducing a fusion gene according to claim 24 into live cells.

30 40. Use of a multi-component detection system according to any one of claims 1 to 22 to monitor protein-protein interactions *in vitro* or *in vivo*.

35 41. Use of a multi-component detection system according to any one of claims 1 to 22 to monitor enzyme activity *in vitro* or *in vivo*.

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42. Use of a multi-component detection system according to any one of claims 1 to 22 to monitor interactions between membrane proteins *in vitro* or *in vivo*.

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43. Use of a multi-component detection system according to any one of claims 1 to 22 to monitor interactions between membrane proteins and non-membrane bound proteins *in vitro* or *in vivo*.

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44. Use of a multi-component detection system according to any of claims 1 to 22 to monitor protein-nucleic acid interactions *in vitro* or *in vivo*.